Role of TNF-α and its 55 and 75 kDa receptors in bronchial hyperreactivity

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Abstract The pathophysiological role of the tumour necrosis factor (TNF) system was studied in adults (n=37) and children (n=43) non asthmatic offspring of asthmatic parents with and without bronchial hyperreactivity proved by methacholine airway challenge test. Serum TNFα and its soluble receptors (sTNF-RI and R2) were determined by enzyme-linked immunosorbent assay (ELISA). Significantly elevated TNFα (adults: mean ± SD=5·18 ± 0·87 pg ml^{-1}, children: 5·08 ± 1·78) *vs.* non-hyperreactives (adults: 4·12 ± 0·43, P<0·0001, children: 3·75 ± 0·68, P=0·0084), sTNF-RI (adults: 1·44 ± 0·31 ng ml^{-1}, children: 1·30 ± 0·25 *vs.* adults: 1·21 ± 0·14, P=0·0305, children: 1·13 ± 0·11 ng ml^{-1}, P=0·0042) and sTNF-R2 (adults: 0·85 ± 0·40 ng ml^{-1}, children: 0·70 ± 0·46 *vs.* adults: 0·56 ± 0·56 P=0·0084, children: 0·33 ± 0·17, P=0·0048) and decreased sTNF-RI/R2 ratio (adults: mean ± SD=0·96 ± 0·73, children: 2·85 ± 2·06 *vs.* adults: 4·82 ± 3·40, P=0·0272, children: 4·42 ± 2·30, P=0·0167) were measured in patients with bronchial hyperreactivity. The provocation doses of methacholine causing a 20% reduction (PD_{20}) in forced expiratory volume in 1 sec (FEV_1) were found to be in a significant negative linear correlation with TNFα, sTNF-RI and R2 levels in hyperreactive adults and with TNFα, sTNF-R2 in hyperreactive children. TNFα correlated significantly with its receptors both in hyperreactive adults and children and with the body mass index (BMI) values of adults. The TNF system may contribute to the pathophysiology of bronchial hyperreactivity. Altered shedding of sTNF-RI seems to occur in hyperreactive patients.

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Keywords TNFα; sTNF-RI; sTNF-R2; bronchial hyperreactivity; bronchial asthma.

INTRODUCTION

Tumour necrosis factor (TNF)α and its soluble receptors (sTNF-RI and sTNF-R2) may have an importance in the pathophysiology of bronchial asthma, as it has been demonstrated in animal (1) and human models (2–4). The cytokine may increase the migration and survival of the eosinophils (5–7) and influence the interaction of cells of the immune system and the bronchial epithelium (8–13). Increased serum concentrations of sTNF-RI and R2 (55 and 75 kDa isoforms of soluble tumour necrosis factor) have been detected in the course of acute asthmatic attack (14). Altered shedding of TNF receptors was detected in auto-inflammatory syndromes (15). The recently characterised membrane metalloprotease ADAM 17 seems to cleave both the receptors and the membrane-bound forms of TNFα and β, however the role of another membrane-bound enzyme was also suggested (16).

The aim of the present study is to evaluate the role of the TNF system, TNFα, soluble TNF-RI and R2 in non-asthmatic relatives of asthmatic patients (adults and children) with and without bronchial hyperreactivitiy.

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METHODS

Patients

Five hundred and twenty-seven childhood asthmatic adults (67·6% male, 32·4% female), previously treated at the 1st Department of Paediatrics, Semmelweis University Budapest, were asked to come to the hospital for a re-examination. 145 adult patients [66·2% male, 33·8% female, age: 37·65 ± 5·65 years (mean ± SD)] and their 142 children (56·5% male, 43·5% female, age: 11·38 ± 5·73 years) were included in the study. The adults were all older than 28 years. According to WHO classification of asthma severity the patients represented all the four

steps (17). The offspring were also examined to reveal their atopic diseases (rhinitis 43%, conjunctivitis 15%, skin manifestations 12%, drug allergy 11%, food allergy 5%, gastrointestinal complaints 4%, non allergic 43%). Allergic prick skin tests were carried out in each case with 12 inhalative allergens (Soluprick, ALK).

The data presented here are part of a follow-up examination. The study was approved by the ethical committee of Semmelweis University. All patients involved in the study gave their written informed consent.

According to spirometric measurements, symptom-free patients with hyperreactive and non-hyperreactive bronchi were chosen for serum TNFα, sTNF-R1 and sTNF-R2 determination. The inclusion/exclusion criteria were to be symptom-free and without any medication for more than 3 years (including inhaled steroid or beta-agonists). There were 37 adults and 43 children examined (Table 1). Their medical history and physical status were recorded on questionnaires.

TNFα, sTNF-R1 and sTNF-R2 measurements were performed in the serum of all the 37 childhood asthmatic adults and in the 43 offspring.

Determination of bronchial hyperreactivity

Aspecific airway challenge tests were performed by increasing doses of methacholine on patients with no pulmonary symptoms. Methacholine of 1% (Lofarma, Milano, Italy) was used in appropriate dilution with distilled water. Pari Provocations Test vaporising equipment was used for the airway provocation. Spirometric measurements were performed using Sensor Medics Vmax apparatus. The patients inhaled vaporised methacholine (0·125, 0·25, 0·5%). A stepwise provocation by inhalation was performed (1·25 l+2·5 l+5·0 l+10·0 l=18·75 l). The patient was allowed to inhale the next concentration of methacholine if symptom-free, and if the reduction of FEV_1 (forced exspiratory volume in the first sec) was less than 20%. Otherwise, the challenge was finished by the inhalation of 18·75 l of the vaporised 0·5% methacholine solution. Spirometric parameters including FVC (forced vital capacity) were registered. Bronchial hyperreactivity was considered at 20% reduction of FEV_1 with methacholine challenge.

The doses of provocation which caused 20% reduction (PD_{20}) of FEV_1 were calculated from the change of spirometric parameters and the amount of methacholine used. In patients without bronchial hyperreactivity, theoretical PD_{20} values were calculated. The PD_{20} values were also compared to the serum concentrations of the cytokine and its receptors.

Since a substantial amount of TNFα can be produced by the fat tissue (18), the patients' body mass indexes (BMI=body weight kg height m^{-2}) were also calculated.

Measurement of serum TNFα and sTNF-R1 and R2

Fasting venous blood samples were collected for TNFα and sTNF-R1 and R2 measurements. Serum samples were centrifuged at 1100 rpm at room temperature and stored at −80°C. The serum concentration of TNFα, (Sigma Immuno Chemicals, St Louis, MO, USA), soluble TNF-R1 and R2 (Bender MedSystems, Austria) were determined using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instruction. The absorption was measured at 450 nm by an ELISA reader. The intra-assay coefficient of variation was 4·2% for TNFα, 1·89 % for sTNF-R1 and 1·4% for sTNF-R2; the inter-assay

Table 1. The clinical and spirometric data of the patients with and without bronchial hyperreactivity

	All patients n=80			
	Adults (n=37)		Children (n=43)	
	Hyperreactives (n=20)	Non-hyperreactives (n=17)	Hyperreactives (n=22)	Non-hyperreactives (n=21)
Male	11	9	14	14
Female	9	8	8	7
Mean age	34 years (29–44 years)	33 years (29–45 years)	14 years (8–21 years)	15 years (8–23 years)
BMI kg m^{-2} mean ± SD	25·76 ± 4·443	25·50 ± 5·286	16·62 ± 2·328	18·53 ± 2·301
CI 95%	23·68–27·84	22·78–28·22	15·58–17·65	17·49–19·58
PD20 FEV_1 mean ± SD	490·9 ± 582·9	17590 ± 12120	265·6 ± 212·1	5462 ± 6981
CI 95%	218·1–763·7	3066–7695	171·5–359·6	1990 ± 8934

coefficient of variation was 7·0, 8·6 and 2·0%, respectively.

The data for TNFα are expressed as pg ml^{-1} and those for sTNF-RI and R2 as ng ml^{-1}.

Statistical analyses

Statistical analyses and graphical illustration of the results was carried out using the Prism 3,0 statistical and graphical program. The Mann–Whitney test was used for comparison of hyperreactive and non-hyperreactive groups. Linear correlation was calculated using the Spearman test between TNFα, sTNF-RI and sTNF-R2 concentrations and clinical parameters of patients; $P < 0{\cdot}05$ was regarded significant.

RESULTS

The patients were divided into bronchial hyperreactive and non-hyperreactive groups according to the decrease of FEV_1 values after airway challenge with methacholine. The clinical data of patients and the PD20 values of spirometric parameters are shown in Table I.

Significantly elevated TNFα concentrations were found in patients with bronchial hyperreactivity (adults: mean ± SD = 5·18 ± 0·87 pg ml^{-1}, children: mean ± SD = 5·08 ± 1·78 pg ml^{-1}) as compared to the non-hyperreactive groups (adults: mean ± SD = 4·12 ± 0·43 pg ml^{-1}, $P < 0{\cdot}0001$, children: mean ± SD = 3·75 ± 0·68 pg ml^{-1}, $P = 0{\cdot}0084$). These data are shown in Fig. 1(a).

Soluble TNF-RI concentrations were also significantly higher both in adults and children with bronchial hyper-

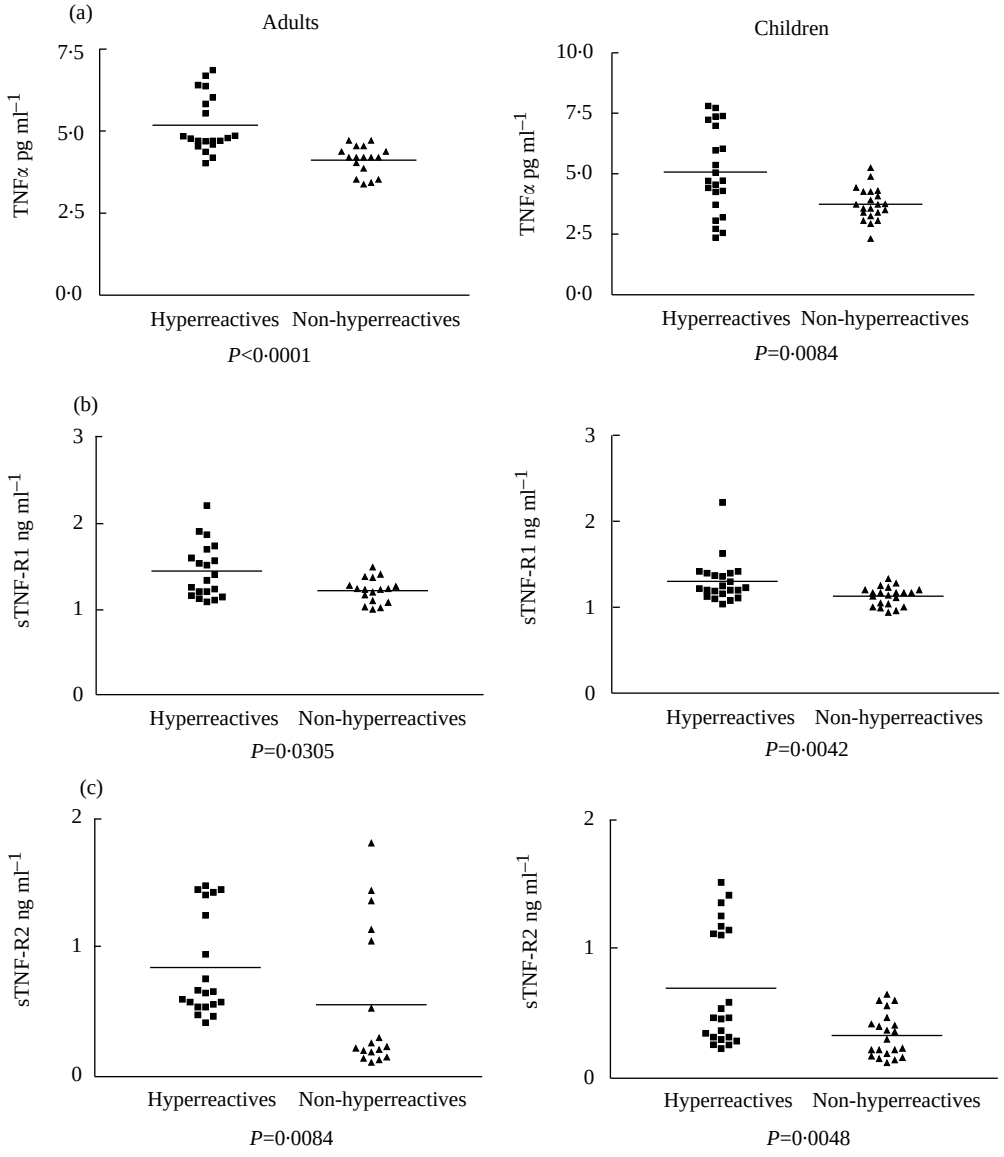

FIG. 1. Serum TNFα, sTNF-RI and sTNF-R2 concentrations in adult and child patients with and without bronchial hyperreactivity. Horizontal bars represent mean values.

reactivity (adults: mean ± SD=1·44 ± 0·31 ng ml^{-1}, children: mean ± SD=1·30 ± 0·25 ng ml^{-1}) as compared to non-hyperreactive patients (adults: mean ± SD=1·21 ±0·14 ng ml^{-1}, P=0·0305, children: mean ± SD=1·13 ± 0·11 ng ml^{-1}, P=0·0042) in [Fig. 1(b)]. Evaluating the soluble TNF-R2 concentrations, we also observed significantly elevated receptor levels both in adult and children bronchial hyperreactive groups (adults: mean ± SD=0·85 ± 0·40 ng ml^{-1} *vs.* mean ± SD=0·56 ± 0·56 ng ml^{-1} P=0·0084, children: mean ± SD=0·70 ± 0·46 ng ml^{-1} *vs.* mean ± SD=0.33 ± 0·17 ng ml^{-1}, P=0·0048) [(Fig. 1(c)].

TNFα and its receptors correlated significantly in all the adult patients (n=37 TNFα–TNF-RI r=0·4361, P=0·0070; TNFα–sTNF-R2 r=0·3708, P=0·0239). Among the hyperreactive children TNFα and sTNF-R2 correlated with each other (children: TNFα–TNF-R2 r=0·6209 P=0·0020) (Table 2).

The ratio of soluble TNF receptors RI/R2 was significantly higher in the non-hyperreactive groups compared to the hyperreactives both in adults and children (hyperreactive adults: mean ± SD=1·96 ± 0·73 compared to non-hyperreactives: mean ± SD=4·82 ± 3·40 P=0·0272, hyperreactive children: mean ± SD=2·85 ± 2·06, non-hyperreactives: mean ± SD=4·42 ± 2·30 P=0·0167) (Fig. 2).

TNFα levels correlated negatively with the PD20 FEV_1 values in both adult and child hyperreactive groups (adults FEV_1: r=−0·4880, P=0·0291; children FEV_1: r=−0·6851 P=0·0004; Table 3). Soluble TNF-RI concentrations were also found to have significant negative linear correlation with the PD 20 doses of FEV_1 (r=−0·4528, P=0·0450) in adult patients. Soluble sTNF-R2 levels showed also a negative linear correlation with the PD20 values of adults (FEV_1: r=−0·6225 P=0·0034) and also in children (r=−0.4340 P=0.0436) (Table 3). The ratio of the soluble TNF receptors RI/R2 positively correlated with that parameter in adults (PD20 FEV_1: r=0·5368, P=0·0147; Table 3).

In adults, TNFα [(Fig. 3(a)] and sTNF-RI [(Fig. 3(b)] showed a significant positive linear correlation with BMI (r=0·3507, P=0·0334 and r=0·4633, P=0·0039 respectively). This was not observed in children. No correlation was found between PD20 FEV_1–BMI,–TNFα/BMI and –sTNF-RI/BMI values of adult hyperreactive patients.

DISCUSSION

We have detected significantly higher TNFα, sTNF-RI and R2 values in symptom-free adult and children patients with bronchial hyperresponsiveness. TNFα concentrations negatively correlated with the provocation doses of methacholine in the airway challenge tests.

TABLE 2. Correlation of TNFα with sTNF-RI and sTNF-R2 levels in patients with hyperreactive and non-hyperreactive bronchi

		Adults		Children	
		Hyperreactives TNFα	Non-hyperreactives TNFα	Hyperreactives TNFα	Non-hyperreactives TNFα
sTNF-RI	r	0·4881	0·0492	0·2477	0·3672
	P	0·0290	0·8513	0·2663	0·1015
sTNF-R2	r	0·6461	0·1455	0·6209	0·0436
	P	0·0021	0·5773	0·0020	0·8510

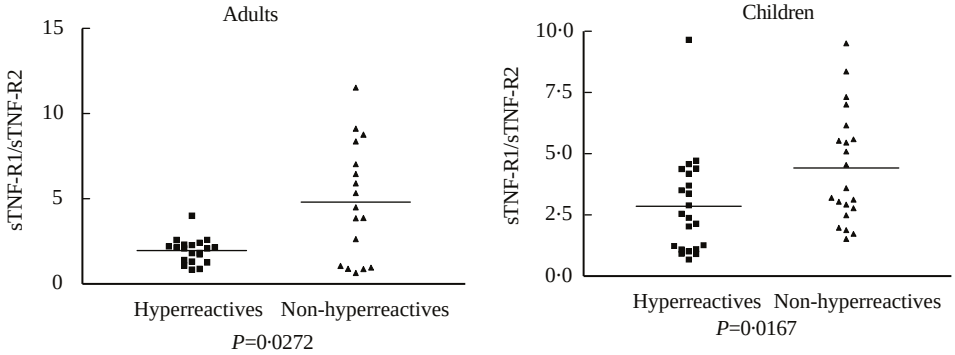

FIG. 2. Serum sTNF-RI/sTNF-R2 values in adult and child patients with and without bronchial hyperreactivity. Horizontal bars represent mean values.

Table 3. Correlation of TNFα, sTNF-RI, sTNF-R2 levels and sTNF-RI/sTNF-R2 ratio with the PD20 values of FEV_1 in patients with hyperreactive bronchi

		Adults PD20 FEV_1	Children PD20 FEV_1
TNFα	r	−0·4880	−0·6851
	P	0·0291	0·0004
STNF-RI	r	−0·4528	−0·1301
	P	0·0450	0·5638
STNF-R2	r	−0·6225	−0·4340
	P	0·0034	0·0436
RI/R2	r	0·5368	0·3829
	P	0·0147	0·0786

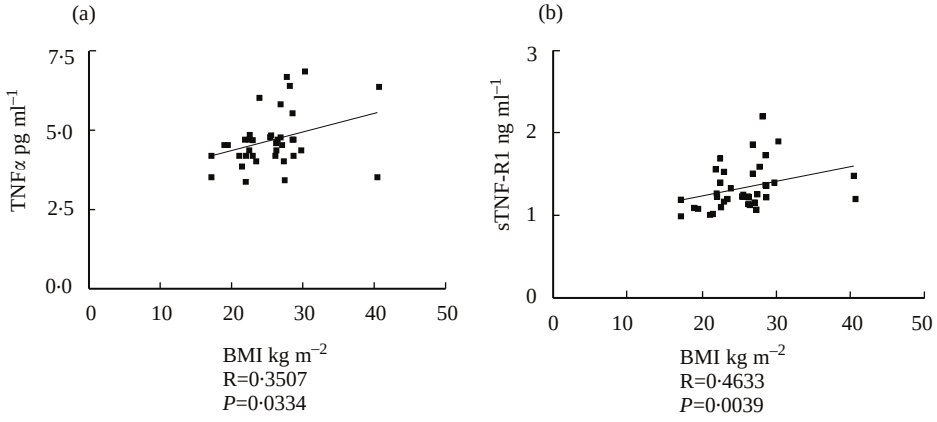

Fig. 3. Correlation of serum TNFα (a) and sTNF-RI (b) values with BMI in adult patients. Horizontal bars represent mean values.

These observations support the role of the activation of the TNF system in the pathomechanism of bronchial hyperreactivity, as it has already been observed in patients with bronchial asthma during acute asthmatic attack (14).

The cellular sources of the elevated cytokine and receptor levels in hyperreactive patients can be the cells of the immune and the broncho-alveolar system. The higher cytokine values may indicate the activated state of these cells (19–21).

Several polymorphisms of the TNF system (-308 G/A promoter and lymphotoxin-α gene *NcoI* polymorphisms) have been observed to be associated with bronchial asthma (4). Alterations in the TNF gene promoter may result in higher production of the cytokine in patients with bronchial hyperreactivity.

The soluble TNF-RI and R2 can serve as physiological competitive antagonists of TNFα. The cytokine can regulate the shedding of its receptors (16). The correlation between TNFα and its soluble receptors may reflect an active counter-regulatory mechanism protecting against the pro-asthmatic effect of the cytokine. The lower sTNF-RI/R2 ratio in hyperreactive patients compared to non-hyperreactive ones may be due to a decreased shedding of sTNF-RI in patients with bronchial hyperresponsiveness. The alteration of sTNF-RI shedding due to the mutation of the extracellular part of the receptor as a cause of an immunological disorder has already been observed in auto-inflammatory syndromes (15).

TNFα and its receptors are also expressed in the adipose cells and elevated TNFα levels have been detected in patients with obesity (18,22).

A correlation of TNFα and sTNF-RI levels with BMI values of patients was observed in our adult patients. This supports the role of the fat tissue as an additional source of the elevated level of TNFα and sTNF-RI.

In conclusion, the contribution of the TNF system can be raised in bronchial hyperresponsiveness. Differential shedding of the soluble TNF receptors may also have a pathophysiological role in this process.

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REFERENCES

1. Ohkawara Y, Lei XF, Stampfli MR, Marshall JS, Xing Z, Jordana M. Cytokine and eosinophil response in the lung, peripheral blood, and bone marrow compartments in a murine model of allergen-induced airways inflammation. *Am J Resp Cell Mol Biol* 1997; **16:** 510–520.
2. Subratty AH, Hooloman NK. Role of circulating inflammatory cytokines in patients during an acute attack of bronchial asthma. *Indian J Chest Dis Allied Sci* 1998; **40:** 17–21.
3. Xu J, Zhong NS. The interaction of tumor necrosis factor alpha and endothelin-1 in pathogenetic models of asthma. *Clin Exp Allergy* 1997; **27:** 568–573.
4. Albuquerque RV, Hayden CM, Palmer LJ, *et al.* Association of polymorphisms within the tumour necrosis factor (TNF) genes and childhood asthma. *Clin Exp Allergy* 1998; **28:** 578–584.
5. Bittleman DB, Erger RA, Casale TB. Cytokines induce selective granulocyte chemotactic responses. *Inflamm Res* 1996; **45:** 89–95.
6. Levi-Schaffer F, Temkin V, Malamud V, Feld S, Zilberman Y. Mast cells enhance eosinophil survival in vitro: role of TNFα and granulocyte-macrophage colony-stimulating factor. *J Immunol* 1998; **160:** 5554–5562.
7. Yamamoto H, Sedgwick JB, Busse WW. Differential regulation of eosinophil adhesion and transmigration by pulmonary microvascular endothelial cells. *J Immunol* 1998; **161:** 971–977.
8. Wallach D, Varfolomeev EE, Malinin NL, Goltsev Yuri V, Kovalenko AV, Boldin MP. Tumor necrosis factor receptor and Fas signaling mechanisms. *Annu Rev Immunol* 1999; **17:** 331–367.
9. Sato M, Takizawa H, Kohyama T. Eosinophil adhesion to human bronchial epithelial cells: regulation by cytokines. *Int Arch Allergy Imm* 1997; **113:** 203–205.
10. Elsner J, Kapp A. Regulation and modulation of eosinophil effector functions. *Allergy* 1999; **54:** 15–26.
11. Krunkosky TM, Fischer BM, Akley NJ, Adler KB. Tumor necrosis factor alpha (TNF-alpha)-induced ICAM-1 surface expression in airway epithelial cells in vitro: possible signal transduction mechanisms. *Ann NY Acad Sci* 1996; **796:** 30–37.
12. Burke-Gaffney A, Hellewell PG. A CD18./ICAM-1-dependent pathway mediates eosinophil adhesion to human bronchial epithelial cells. *Am J Resp Cell Mol Biol* 1998; **19:** 408–418.
13. Hirata N, Kohrogi H, Iwagoe H, *et al.* Allergen exposure induces the expression of endothelial adhesion molecules in passively sensitised human bronchus: time course and the role of cytokines. *Am J Resp Cell Mol Biol* 1998; **18:** 12–20.
14. Yoshida S, Hashimoto S, Nakayama T, Kobayashi T, Koizumi A, Horie T. Elevation of serum soluble tumor necrosis factor (TNF) receptor and IL-1 receptor antagonist levels in bronchial asthma. *Clin Exp Immunol* 1996; **106:** 73–78.
15. Galon J, Aksentijevich I, McDermott MF, O'Shea JJ, Kastner DL. TNFRSF1A mutations and autoinflammatory syndromes. *Curr Opin Immunol* 2000; **12:** 479–486.
16. Dri P, Gaparini C, Menegazzi R, *et al.* TNF-induced shedding of TNF receptors in human polymorphonuclear leukocytes: role of the 55-kDa TNF receptor and involvement of a membrane-bound and non-matrix metalloproteinase. *J Immunol* 2000; **165:** 2165–2172.
17. Global Initiative for Asthma. Pocket guide for asthma management and prevention. Bethesda, MD: NHLBI/WHO. Revised edition 1998.
18. Winkler G, Lakatos P, Salamon F, *et al.* Elevated serum TNF-alpha level as a link between endothelial dysfunction and insulin resistance in normotensive patients with android type obesity. *Diabetic Med* 1999; **16:** 207–211.
19. Matsuyama G, Ochiai K, Ishihara C, Kagomi M, Tomioka H, Koya N. Heterogeneous expression of tumor necrosis factor-alpha receptors Iand II on human peripheral eosinophils. *Int Arch Allergy Imm* 1998; **117:** 28–33.
20. Anticevich SZ, Hughes JM, Black JL, Armour CL. Induction of human airway hyperresponsiveness by tumour necrosis factor-alpha. *Eur J Pharmacol* 1995; **284:** 221–225.
21. Laan MP, Koning H, Baert MRM, *et al.* Levels of soluble intercellular adhesion molecule-1, soluble E-selectin, tumor necrosis factor-α, and soluble tumor necrosis factor receptor p55 and p75 in atopic children. *Allergy* 1998; **53:** 51–58.
22. Hotamisligil GS. Mechanism of TNF-α induced insulin resistance. *Exp Clin Endocr Diab* 1999; **107:** 119–125.